PATENTS
Atty. Docket No. HYZ-030CPCN3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Agrawal, et al.	Art Unit:	1635
Serial No.:	09/777,526	Examiner:	Gibbs, T
Filing Date:	February 6, 2001		
Title:	**Method of Down-Regulating Gene Expression**		

Commissioner for Patents
Washington, DC 20231

DECLARATION OF DR. RUIWEN ZHANG UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Ruiwen Zhang, declare as follows:

1. I am a co-inventor of the invention claimed in the above-referenced patent application.

2. I obtained my M.D. in 1983, my Ph.D. in Toxicology and Occupational Epidemiology in 1988, and was a post-doctoral fellow/clinical pharmacology fellow for three years at the University of Alabama at Birmingham. I have been involved in oligonucleotide research, including antisense oligonucleotide research, since 1992. I have authored or co-authored more than 100 full-length journal articles, book chapters, and reviews, and 90 abstracts. I have edited a book about antisense oligonucleotide therapeutics, and have given numerous invited oral presentations concerning antisense oligonucleotide synthesis and therapy. I hold at least two issued U.S. patents in the field of antisense oligonucleotides. My curriculum vitae along with a list of these publications and presentations is enclosed herewith as Appendix A.

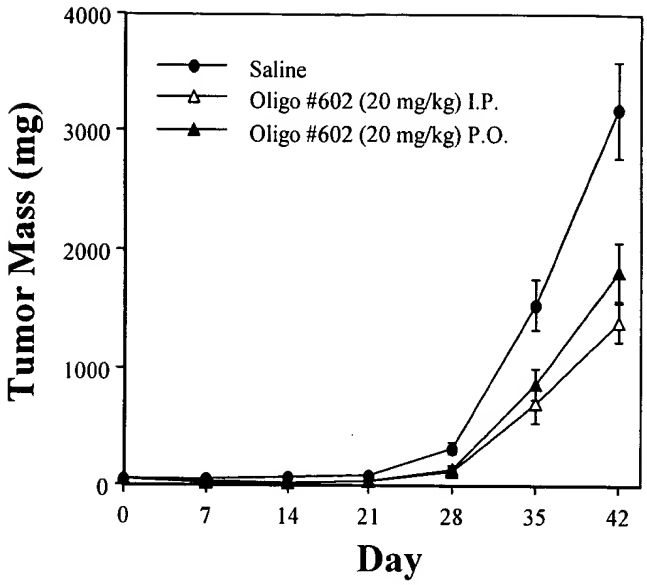
Considered
6/20/03

3. Currently, I am Associate Professor of Clinical Pharmacology, Pharmacology and Toxicology and Director, Cancer Pharmacology Laboratory at the University of Alabama at Birmingham School of Medicine.

4. I am familiar with the Office Action dated October 8, 2002, in which the Examiner stated that the "specification as filed does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims" (Office Action, page 4). The Office Action further states that "undue experimentation would have been required by one skilled in the art at the time the application was filed to practice the claimed invention using *any* type of oligonucleotide claimed, except for an all phosphorothioate oligonucleotide having at least two 2'-O-methyl ribonucleotides at each end" (pages 5-6).

5. I disagree with these statements.

6. Additional experiments have been performed using oligonucleotide # 602 in which oral administration of this oligonucleotide resulted in a decreased tumor mass in nude mice into which tumor cells had been injected compared to the tumor mass in mice to which had been administered a saline control. The sequence of oligo # 602 is 5'-**UG**ACACCTGTTCTCAC**UCAC**-3'. Nucleotides shown in normal are 2'-deoxyribonucleotides, and nucleotides shown in bold face, underlined are 2'-O-methylribonucleotides. This oligonucleotide was administered via gavage at a dose of 20 mg/kg, 5 days a week for 6 weeks. Data from these experiments is as follows:



7. This data shows that an oligonucleotide with two 2′-O-methylribonucleotides at the 5′ terminal end and four 2′-O-methylribonucleotides at the 3′ terminal end is effective in decreasing tumor mass when administered orally relative to the control, and therefore, would have been present in intact form in plasma at least six hours following oral administration.

8. Additionally, a chapter of a peer-reviewed book, Agrawal and Zhang, "Pharmacokinetics and Bioavailability of Antisense Oligonucleotides Following Oral and Colorectal Administrations in Experimental Animals" in Handbook of Experimental Pharmacology, Vol. 131, Antisense Research and Application (Stanley T. Crooke ed.) Springer Verlag 1998, attached hereto as Appendix B, discloses the oral bioavailability of an oligonucleotide MBO-2. MBO-2 contains 4 methylphosphonate linkages at both the 3′ and 5′ terminal ends of a phosphorothioate oligonucleotide (Page 527). The article states that "[t]he stability of MBO-2 in the contents of the stomach and small and large intestines of mice following oral gavage (30 mg/kg) showed the presence of mainly intact MBO-2 in the contents of the small and large intestine (Fig 3B). . . . Analysis of the extracted radioactivity from plasma and various tissues by

PAGE and HPLC showed the presence of both intact and degraded forms of MBO-2 (Fig. 4B)." (Page 533)

9. This data shows that an oligonucleotide with four methylphosphonate linkages at both the 3' and 5' terminal ends exhibits oral bioavailability and was present in intact form in plasma at least six hours following oral administration.

10. Accordingly, the specification of the patent application provides sufficient support such that one with skill in oligonucleotide chemistry, using the teachings of the specification, could perform the claimed method without undue experimentation. This fact is corroborated by the data described herein.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 03/31/03

Ruiwen Zhang